PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 145 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/13907	International filing date *(day/month/year)* 08.12.2003	Priority date *(day/month/year)* 06.12.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant INNOGENETICS N.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01.07.2004	16.09.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Helliot, B Telephone No. +49 89 2399-7793

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/13907

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-6, 8-23, 25, 26, 28-38	as originally filed
24, 27	filed with telefax on 01.04.2005
7	received on 12.07.2005 with letter of 08.07.2005

Claims, Numbers

1-15	received on 12.07.2005 with letter of 08.07.2005

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-2,4-6,8,11,13-14
	No:	Claims	3,7,9-10,12,15
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

ITEM I:

The sequence listing furnished with the letter dated of March 29 2001, does not form part of the international application (Rule 13ter.1(f) PCT.

ITEM V:

1. INTRODUCTION

The following documents (D1-D2) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: WO-A-96/00298 (cited in the application)

D2: US-A-6312903

The present application relates to a method for the specific detection and/or identification of *Staphylococcus species,* in particular of *S. aureus*, using nucleic acid sequences derived from the ITS (Internal Transcribed Spacer) region.

2. NOVELTY (Art. 33(2) PCT)

a. No cited prior-art document discloses isolated nucleic acid molecules consisting of SEQ ID N° 1 or of SEQ ID N° 2. Therefore, the subject-matter of **independent claim 1** and that of **claim 2**, both relating to an isolated nucleic acid molecule, are considered novel in the sense of Art. 33(2) PCT.

b. No cited prior-art document discloses a set of two polynucleotide probes, said two probes hybridizing specifically to SEQ ID N° 1 or SEQ ID N° 2, wherein there are no more than 25 nucleotides between said two probes. Therefore, the subject-matter of **claims 5-6**, relating to a set of polynucleotide probes, is considered novel in the sense of Art. 33(2) PCT.

c. For the same reasons as set out under items 2-a and 2-b and because no prior-art document discloses the use of an isolated nucleic acid molecules consisting of SEQ ID N° 1 or SEQ ID N° 2 for the detection and identification of *Staphylococcus* species, the subject-matter of **independent claim 8** is considered novel in the sense of Art. 33(2) PCT.

d. However, the IPEA is still of the opinion that the present application does not meet the requirements of Art. 33(2) PCT, because the subject-matter of **claims 3, 7, 9-10, 12, 15** is not new over D1 for the following reasons.

D1, which is cited by the Applicant (see p. 6, l. 18), relates to nucleic acid probes derived from the spacer region between the 16S and 23S ribosomal ribonucleic acid (rRNA) genes, to be used for the specific detection of eubacterial organisms in a biological sample by a hybridization procedure, as well as to nucleic acid primers to be used for the amplification of said spacer region of eubacterial organisms in a biological sample wherein:

i) the nucleic acid sequence SEQ ID N° 53 exhibits 100% identity in 30 nucleotide overlap with the nucleic acid sequence SEQ ID N° 1 of the present application (p. 41, l. 8; relevant against **claims 3, 7 and 10**);

ii) the nucleic acid sequence SEQ ID N° 53 is used as probe for the detection and identification of *Staphylococcus* species in a sample (p. 41, l. 5-25 and p. 101, l. 4-6; relevant for **claims 7, 9 and 10**);

iii) a method for the detection and identification of at least one micro-organism, or for the simultaneous detection of several micro-organisms in a sample, comprising the steps of: (a) if need be releasing, isolating and/or concentrating the polynucleic acids from the micro-organism(s) to be detected in the sample; (b) if need be amplifying the 16S-23S rRNA spacer region, or a part of it, from the micro-organism(s) to be detected, with at least one suitable primer pair; (c) hybridizing the polynucleic acids of step (a) or (b) with a set of probes comprising at least two probes under the same hybridization and wash conditions, with said probes being selected from the sequences of table Ia or equivalents thereof, and/or from taxon-specific probes derived from any of the spacer sequences as represented in figures 1-103, with said taxon-specific probe being selected such that it is capable of hybridizing under the same hybridization and wash conditions as at least one of the probes of table Ia; (d)

detecting the hybrids formed in step (c); (e) identification of the micro-organism(s) present in the sample from the differential hybridization signals obtained in step (d) (claim 1; relevant against **claims 10 and 12**);
iii) a kit for the detection and identification of at least one micro-organism, or the simultaneous detection and identification of several micro-organisms in a sample, comprising the following components (a) when appropriate, at least one suitable primer pair to allow amplification of the intercistronic 16S-23S rRNA spacer region, or a part of it; (b) at least one of the probes as defined above; (c) a buffer, or components necessary to produce the buffer, enabling a hybridization reaction between said probes and the polynucleic acids present in the sample, or the amplified products thereof (p. 48, l. 10-22; relevant against **claim 15**).

The IPEA does not share the opinion of the Applicant according to which, the probe listed as SEQ ID N° 53 in D1 is not suitable for the identification of *Staphylococcus* species (see replies of the Applicant, dated April, 1st 2005 and July, 8th 2005) because D1 discloses that the nucleic acid sequence SEQ ID N° 53 is used as probe for the detection and identification of all *Staphylococcus* species in a sample (p. 41, l. 5-25 and p. 101, l. 4-6).

Moreover, the IPEA is still of the opinion that, even if the present claim 3 mentions the particular detection and identification of S. aureus, the scope of said claim 3 relates broadly to probes for the detection and identification of *Staphylococcus* species, since the term "in particular" has no limiting value. Thus, the Examining Division does not acknowledge the arguments of the Applicant with respect the specificity of the probes to which claim 3 refers.

Thus, the IPEA is still convinced that the subject-matter of **claims 3, 7, 9-10, 12, 15** is not new over D1. The present application does, therefore, not meet the requirements of Art. 33(2) PCT.

For same reasons as set out herein above under the item 2-e, D2 which relates to nucleic acid probes derived from the spacer region between the 16S and 23S ribosomal ribonucleic acid (rRNA) genes, to be used for the specific detection of eubacterial organisms in a biological sample by a hybridization procedure, as well

as to nucleic acid primers to be used for the amplification of said spacer region of eubacterial organisms in a biological sample wherein the nucleic acid sequence SEQ ID N° 53 exhibits 100% identity in 30 nucleotide overlap with the nucleic acid sequence SEQ ID N° 1 of the present application (Tab. 1-a, c. 56) is novelty destroying for the **claims 3, 7, 9-10, 12 and 15**.

3. INVENTIVE STEP (Art. 33(3) PCT)

a. Moreover, the IPEA is not convinced by the arguments of the Applicant and is still of the opinion that the subject-matter of **claim 1** does not involve an inventive step (Art. 33(3) PCT).

In its reply, dated April, 1st 2005, the Applicant argues that "one of the probes described in D1/D2 falls accidentally in the useful ITS region identified in the present invention. The IPEA does not share this opinion because the aim of D1/D2 is identical as that of the present application which is the selection of probes or sets of probes, which have as target the 16S-23S rRNA spacer region, and which allow the detection and identification of at least one, and preferably more than one, of the above mentioned microorganisms, among them Staphylococcus species and more particularly *S. aureus* and *S. epidermidis* (p. 3, l. 9-15 and p. 4, l. 22-23).

Moreover, D1 discloses a 383 nucleic acid sequence, listed as SEQ ID N° 139, which represents the DNA sequence of one of the 16s-23s spacer region from *Staphylococcus aureus* (Fig. 64). The said sequence is used in a method for the detection and identification of at least one micro-organism, or for the simultaneous detection of several micro-organisms in a sample (claim 1 and p. 41, l. 5-25). The nucleic acid sequence, listed as SEQ ID N° 139, exhibits 100% identity in 143 nucleotide overlap with the nucleic acid sequence, listed as SEQ ID N° 1 of the present application.

Moreover, the Applicant argues that a great advantage of the application is that "the set of probes yields a 100% specificity for the identification of *S. aureus*, *S. epidermis* and *S. haemolyticus*" while "the probe STAU-ICG1 of D1/D2 has a specificity of 0% for the various species". The IPEA does not share this view since

in the application, the high specificity of the detection of *S. aureus*, *S. epidermis* and *S. haemolyticus* is obtained with the particular set of probes listed as SEQ ID N° 17 and 19 (see p. 36, l. 13-14) and since the present application is silent as to any other high detection specificity obtained with other sets of probes. Moreover, the IPEA refutes this argument because in D1/D2, the probe STAU-ICG1 reacts with all *Staphylococcus* species (see Example 9 and Tab. 10).
Thus, in view of the identity of aims between the present application and D1/D2 and in view of the nucleic acid sequence, listed as SEQ ID N° 139, the subject-matter of **claim 1** differs from that of D1/D2, which is considered as the closest prior art, in that the nucleic acid sequence SEQ ID N° 1 is shorter than the one disclosed in D1/D2.

Thus, in view of the arguments as set out herein above, and since the present application is silent as to any data showing an improvement of the method of detection/identification when using the nucleic acid sequence SEQ ID N° 1 in comparison with the detection/identification method of D1 or as to any other surprising effect linked to the nucleic acid sequence SEQ ID N° 1, present nucleic acid sequence SEQ ID N° 1 appears to be an arbitrary choice and it would be then obvious for the skilled person to design a 143 nucleic acid sequence from the nucleic acid sequence SEQ ID N° 139 of D1.

The subject-matter of **claim 1** does, therefore, not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

b. For the same reasons as set out under item 3-b herein above and since the nucleic acid sequence listed as SEQ ID N° 2 exhibits 100% identity with the sequence listed as SEQ ID N° 139 in D1, the subject-matter of **claim 2** does not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

c. The subject-matter of **claim 4** does not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT), because it is common practice to design primers from a nucleic acid sequence. Since the nucleic acid sequences SEQ ID N° 1 or SEQ ID N° 2 do not involve any inventive step (see item 3-a and 3-b), a primer designed from these sequences is not inventive.

d. The FRET hybridization probe test is well known in the art for all kinds of homogeneous hybridization assays (see present description p. 12, l. 20-23), and requires a set of probes wherein the probes are located very close to each other, i.e. wherein the gap between the two hybridization probes is as small as possible, and notably consists of about 0 to 25 bases (see claim 1 of WO97/46707; cited by the Applicant on p. 13, l. 8). Since the nucleic acid sequences SEQ ID N° 1 or SEQ ID N° 2 are not inventive (see item 3-a and 3-b) and since the Applicant is award that the gap between the hybridized probes has to be as small as possible, it would be then obvious for the skilled person, to design a set of probes which hybridizes to the nucleic acid sequences SEQ ID N° 1 or SEQ ID N° 2 and wherein there are no more than 25 nucleotides between said two probes, The subject-matter of **claim 5** does, therefore, not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

e. Even if the IPEA acknowledges a surprising effect linked to the use of the particular set of probes listed as SEQ ID N° 17 and 19 (see item 3-a), the actual scope of the present **claim 6** is not restricted to the sole set of probes listed as SEQ ID N° 17 and 19. The scope of **claim 6** is, thus, as broad as to encompass other sets of probes for which no data showing a particular technical effect in the detection and identification of a particular *Staphylococcus* is provided. In view of the fact that the set of probes as disclosed in the present claim 5, does not involve an inventive step (see item 3d herein above), the different sets of probes disclosed in the present **claim 6** represent obvious alternatives of probes. The subject-matter of **claim 6** does, therefore, not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

f. For the same reasons as set out under items 3-a and 3-b herein above, the subject-matter of **claim 8**, which relates to the use of a nucleic acid molecule consisting of SEQ ID N° 1 and SEQ ID N°2 for the detection and identification of *Staphylococcus* species, does not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

Moreover, D1 discloses the use of the nucleic acid sequence SEQ ID N° 139 and probes derived from the said sequence for the detection and identification of one or more *Staphylococcus* species in a sample. Since the nucleic acid sequence

SEQ ID N° 139 encompass the nucleic acid sequence SEQ ID N° 1 of the present invention and since the use of the nucleic acid sequence SEQ ID N° 1 and SEQ ID N° 2 for *Staphylococcus* species detection in a sample does not lead to any surprising effect, it would be then obvious for the skilled person to use alternative nucleic acid sequences designed from the nucleic acid sequence SEQ ID N° 139 to detect or to identify *Staphylococcus* species in a sample, thereby arriving at the use of **claim 8**. The subject-matter of **claim 8** does, therefore, not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

g. Moreover, in absence of any surprising technical effect linked to the use of the different primer pairs as disclosed in the **claim 11**, and since D1 discloses a method for the detection and identification of at least one micro-organisms species, or for the simultaneous detection of several micro-organisms in a sample, wherein the 16S-23S rRNA spacer region is amplified with at least one suitable primer pair (see claim 1), it would be then obvious for the skilled person to use alternative set of primers for performing the method for the detection and identification of at least one micro-organisms species, or for the simultaneous detection of several micro-organisms in a sample, as disclosed in D1, thereby arriving at the method of **claim 11**.
The subject-matter of **claim 11** does, therefore, not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

h. For the same reasons as set out under item 3-a, 3-b and 3-d, the subject-matter of **claim 13-14** does, therefore, not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

Table1

SEQ IDs	Use	References	Length	Sequence
SEQ ID NO 1				TTTGTACATTGAAXACTAGATAAGTAAGTAXAATATAGATTTTACCAYGCAAAACCGAGTGAATA AAGYGTTTTYAAZAAGCWTGAATTCATAAVAAATAATCGZTAGZGTTCGAYAGAACACTCACAA GUTTAATAACWSGT
SEQ ID NO 2				TTTGTACATTGAAAACTAGATAAGTAAGTAAAATATAGATTTTACCAAGCAAAACCGAGTGAATA AAGAGTTTTAAATAAGCTTGAATTCATAAGAAATAATCGCTAGTGTTCGAAAGAACACTCACAA GATTAATAACGCGT
SEQ ID NO 3	CO	RStau6WTcompl.1b	63	ACGCTCACATACGGCTTCGTTTTCATTATTTTAAATGCTCATTTACATAAGTAAACTCTGCTT
SEQ ID NO 4	CO	WToligoHP5	69	CTTATGAATTCAAGCTTATTTAAAACTCTTTATTCACTCGGTTTTGCTTGGTAAAATCTATATTTT ACT
SEQ ID NO 5	CO	WToligoHP6	70	CGAACACTAGCGATTATTTCTTATGAATTCAAGCTTATTTAAAACTCTTTATTCACTCGGTTTTG CTTGG
SEQ ID NO 6	CO	RStau7WTcompl.1b	81	TTATAAGTCAAACGTTAACATGAAGTTACGTTCTTTTATAAAAAGATTTAAACGCGTTATTAATCT TGTGAGTGTTCTTTC
SEQ ID NO 7	CO	RStau6WTcompl.1a	83	TATAAGTCAAACGCTCACATACGGCTTCGTTTTCATTATTTTAAATGCTCATTTACATAAGTAAA CTCTGCTTTAAAATAATT
SEQ ID NO 8	CO	RStauHP1WTCompl.1	88	CTTATTTAAAACTCTTTATTCACTCGGTTTTGCTTGGTAAAATCTATATTTTACTTACTTATCTAG TTTTCAATGTACAAATAATGGT
SEQ ID NO 9	CO	WToligo-StauHP4	88	ATTTAAAACTCTTTATTCACTCGGTTTTGCTTGGTAAAATCTATATTTTACTTACTTATCTAGTTTT CAATGTACAATTTCTTTTTAG
SEQ ID NO 10	CO	RStauHP2WTcompl.1	89	GTGAGTGTTCTTTCGAACACTAGCGATTATTTCTTATGAATTCAAGCTTATTTAAAACTCTTTATT CACTCGGTTTTGCTTGGTAAAAT
SEQ ID NO 11	CO	RStauHP2cWtcompl.1	89	ATTTTACCAAGCAAAACCGAGTGAATAAAGAGTTTTAAATAAGCTTGAATTCATAAGAAATAATC GCTAGTGTTCGAAAGAACACTCAC
SEQ ID NO 12	CO	RStauHP3WTcompl.1	91	TTTAAACGCGTTATTAATCTTGTGAGTGTTCTTTCGAACACTAGCGATTATTTCTTATGAATTCA AGCTTATTTAAAACTCTTTATTCACT
SEQ ID NO 13	CO	RStau7WTcompl.1a	101	TCCACCATTTTTATAAGTCAAACGTTAACATGAAGTTACGTTCTTTTATAAAAAGATTTAAACGC GTTATTAATCTTGTGAGTGTTCTTTCGAACACTAGC
SEQ ID NO 14	HP	RStaphSP-5LC6.1	21	AGATTTTACCAAGCAAAACCG
SEQ ID NO 15	HP	RStauHP5-3Fl.1	21	AGATTTTACCAAGCAAAACCG
SEQ ID NO 16	HP	RStauHP6a-5LC6.1	21	AAGCTTGAATTCATAAGAAAT
SEQ ID NO 17	HP	RStauHP6-3Fl.1	23	CCGAGTGAATAAAGAGTTTTAAA
SEQ ID NO 18	HP	RStauHP4-5LC6.1	24	CCAAGCAAAACCGAGTGAATAAAG
SEQ ID NO 19	HP	RStauHP6b-5LC6.1	24	GCTTGAATTCATAAGAAATAATCG
SEQ ID NO 20	HP	RStauHP5b-5LC6.1	25	GAATAAAGAGTTTTAAATAAGCTTG
SEQ ID NO 21	HP	RStauHP5a-5LC6.1	27	GTGAATAAAGAGTTTTAAATAAGCTTG
SEQ ID NO 22	HP	RStauHP9-5LC6	27	AAGCTTGAATTCATAAGAAATAATCGC
SEQ ID NO 24	HP	RStau63Fl.1	29	AAGCAGAGTTTACTTATGTAAATGAGCAT
SEQ ID NO 25	HP	RStauHP1-5LCR64.1	29	TACCAAGCAAAACCGAGTGAATAAAGAGT
SEQ ID NO 26	HP	RStaphSP-3Fl.1	29	TTTGTACATTGAAAACTAGATAAGTAAGT
SEQ ID NO 27	HP	RStauHP7-3Fl	29	GCAAAACCGAGTGAATAAAGAGTTTTAAA
SEQ ID NO 28	HP	RStauHP7a-5LCR6	30	AAGCTTGAATTCATAAGAAATAATCGCTAG
SEQ ID NO 29	HP	RStauHP9-3Fl	30	AGCAAAACCGAGTGAATAAACAGTTTTAAA
SEQ ID NO 30	HP	RStauHP4-3Fl.1	31	ATTGTACATTGAAAACTAGATAAGTAAGTAA
SEQ ID NO 31	HP	RStauHP3-LC6.1	32	AGTGTTCGAAAGAACACTCACAAGATTAATAA
SEQ ID NO 32	HP	RStauHP8.3Fl	32	AGCTTATTTAAAACTCTTTATTCACTCGGTTT
SEQ ID NO 33	HP	RStau65LC6.1	33	TAAAATAATGAAAACGAAGCCGTATGTGAGCGT
SEQ ID NO 34	HP	RStau65LC7.1	33	TAAAATAATGAAAACGAAGCCGTATGTGAGCGT
SEQ ID NO 35	HP	RStauHP2-LC6.1	33	GAATTCATAAGAAATAATCGCTAGTGTTCGAAA
SEQ ID NO 36	HP	RStauHP2c-3Fl.1	33	TTTCGAACACTAGCGATTATTTCTTATGAATTC
SEQ ID NO 37	HP	RStauHP2-3Fl.1	34	GCAAAACCGAGTGAATAAAGAGTTTTAAATAAGC
SEQ ID NO 38	HP	RStauHP2c-LC6.1	34	GCTTATTTAAAACTCTTTATTCACTCGGTTTTGC
SEQ ID NO 39	HP	RStauHP8.5LCR6	36	GCTTGGTAAAATCTATATTTTACTTACTTATCTAGT
SEQ ID NO 40	HP	RStauHP1-3Fl.1	38	GTACATTGAAAACTAGATAAGTAAGTAAAATATAGATT
SEQ ID NO 41	HP	RStauHP3-3Fl.1	38	GAGTTTTAAATAAGCTTGAATTCATAAGAAATAATCGC
SEQ ID NO 42	HP	RStau73Fl.1	39	GAAAGAACACTCACAAGATTAATAACGCGTTTAAATCTT
SEQ ID NO 43	HP	RStau75LC6.1	41	TTATAAAAGAACGTAACTTCATGTTAACGTTTGACTTATAA
SEQ ID NO 44	HP	RStau75LC7.1	41	TTATAAAAGAACGTAACTTCATGTTAACGTTTGACTTATAA
SEQ ID NO 45	Pr	RStauFP18.1	17	CTTCAGAAGATGCGGAA
SEQ ID NO 46	Pr	RStauRP22.1	18	TTTCGAACACTAGCGATT
SEQ ID NO 47	Pr		20	GTTATTAAUCTTGTGAGTGTT
SEQ ID NO 48	Pr		20	ACSXGTTATTAAUCTTGTGAG
SEQ ID NO 49	Pr	RStauFP19.1	19	CTTCAGAAGATGCGGAATA
SEQ ID NO 50	Pr	RStauFP20.1	19	TTCTTCAGAAGATGCGGAA
SEQ ID NO 51	Pr	Staph-P26rev	19	TTCTTTCGAACACTAGCGA
SEQ ID NO 52	Pr	StauP01	20	ACCAAGCAAAACCGAGTGAA
SEQ ID NO 53	Pr	Staph-P25rev	20	GTTCTTTCGAACACTAGCGA
SEQ ID NO 54	Pr		20	SXGTTATTAAUCTTGTGAGTG
SEQ ID NO 55	Pr	StaphP28rev	20	CGCGTTATTAATCTTGTGAG

<SEQ ID NO:16;DNA;staphylococcus>
AAGCTTGAATTCATAAGAAAT

<SEQ ID NO:17;DNA;staphylococcus>
CCGAGTGAATAAAGAGTTTTAAA

<SEQ ID NO:18;DNA;staphylococcus>
CCAAGCAAAACCGAGTGAATAAAG

<SEQ ID NO:19;DNA;staphylococcus>
GCTTGAATTCATAAGAAATAATCG

<SEQ ID NO:20;DNA;staphylococcus>
GAATAAAGAGTTTTAAATAAGCTTG

<SEQ ID NO:21;DNA;staphylococcus>
GTGAATAAAGAGTTTTAAATAAGCTTG

<SEQ ID NO:22;DNA;staphylococcus>
AAGCTTGAATTCATAAGAAATAATCGC

<SEQ ID NO:24;DNA;staphylococcus>
AAGCAGAGTTTACTTATGTAAATGAGCAT

<SEQ ID NO:25;DNA;staphylococcus>
TACCAAGCAAAACCGAGTGAATAAAGAGT

<SEQ ID NO:26;DNA;staphylococcus>
TTTGTACATTGAAAACTAGATAAGTAAGT

<SEQ ID NO:27;DNA;staphylococcus>
GCAAAACCGAGTGAATAAAGAGTTTTAAA

<SEQ ID NO:28;DNA;staphylococcus>
AAGCTTGAATTCATAAGAAATAATCGCTAG

<SEQ ID NO:29;DNA;staphylococcus>
AGCAAAACCGAGTGAATAAACAGTTTTAAA

<SEQ ID NO:30;DNA;staphylococcus>
ATTGTACATTGAAAACTAGATAAGTAAGTAA

<SEQ ID NO:31;DNA;staphylococcus>
AGTGTTCGAAAGAACACTCACAAGATTAATAA

<SEQ ID NO:32;DNA;staphylococcus>
AGCTTATTTAAAACTCTTTATTCACTCGGTTT

<SEQ ID NO:33;DNA;staphylococcus>
TAAAATAATGAAAACGAAGCCGTATGTGAGCGT

<SEQ ID NO:34;DNA;staphylococcus>
TAAAATAATGAAAACGAAGCCGTATGTGAGCGT

<SEQ ID NO:35;DNA;staphylococcus>

This particular region of the ITS, also referred to as the "target region" or "target sequence", can be defined as a nucleic acid molecule consisting of SEQ ID NO 1 or SEQ ID NO 2, or as a nucleic acid molecule that is homologous to SEQ ID NO 1 or 2, their RNA form wherein T is replaced by U, or their complementary form.

This term "target sequence" covers all the homologous sequences found in the ITS of any *Staphylococcus* species, said homologous sequences are also referred to herein after as "homologues". The degree of homology is then higher than 75%, generally higher than 80%, and even higher than 90%.

In the framework of this invention, "homologues" are then homologous sequences to SEQ ID NO 1 or 2 or to any fragment thereof, localized in the ITS region of any Staphylococcus species, SEQ ID NO 1 and 2 being derived from different *S. aureus* strains.

New polynucleotides for use as probes and/or primers designed from the target sequence of the invention for the detection and/or identification of *Staphylococcus* species are also an object of the invention.

In other words, an object of the invention relates to new polynucleotides for use as probes and/or primers, which hybridize with the target sequence of the invention for the detection and/or identification of *Staphylococcus* species.

In particular, an object of the invention is an isolated nucleic acid molecule that specifically hybridizes to SEQ ID NO 1 or 2, or to the RNA form of said SEQ ID NO 1 or 2 wherein T is replaced by U, or to the complementary form of said SEQ ID NO 1 or 2, or to a fragment of at least 20 contiguous nucleotides thereof, or to any of their homologues, for the detection and identification of *Staphylococcus* species, in particular of *S. aureus*, with the proviso that SEQ ID NO4977 of EP786519 is excluded. This sequence has just been mentioned as found in the genome of *Staphylococcus aureus* without any function indicated.

Preferred polynucleotide probes are between about 5 to about 50 bases in length, more preferably from about 10 to about 25 nucleotides and are sufficiently homologous to the target sequence.

Polynucleotides of SEQ IDs NO 1 to 70 and any of their homologues may be used as probes.

Preferred probes are polynucleotides of SEQ IDs NO 14, 16 to 22, 25 to 32, 35 to 42 and homologues.

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CLAIMS

1. An isolated nucleic acid molecule consisting of SEQ ID NO 1, its complementary form, or RNA form thereof.

2. An isolated nucleic acid molecule consisting of SEQ ID NO 2, its complementary form, or RNA form thereof.

3. An isolated nucleic acid molecule that specifically hybridizes to SEQ ID NO 1 or 2, or to the RNA form of said SEQ ID NO 1 or 2 wherein T is replaced by U, or to the complementary form of said SEQ ID NO 1 or 2, or to a fragment of at least 20 contiguous nucleotides thereof, or to any of their homologues, for the detection and identification of a *Staphylococcus* species, in particular of *S. aureus*, with the proviso that the following nucleic acid molecule is excluded:

- SEQ ID NO 4977 of EP 786519.

4. An isolated nucleic acid molecule according to claim 3 consisting of a nucleic acid selected from the group consisting of SEQ IDs NO 14, 16 to 22, 25 to 32, 35 to 42, 51, 52, 53, 55, 58, 65, 67, 68, 69 and 70.

5. A set of two polynucleotide probes, said two probes hybridizing specifically to SEQ ID NO 1 or SEQ ID NO 2 or homologues, or to their RNA form wherein T is replaced by U, or to their complementary form, wherein there are no more than 25 nucleotides between said two probes.

6. A set of two polynucleotide probes according to claim 5 consisting of SEQ IDs NO 15 and 20, or SEQ IDs NO 15 and 21, or SEQ IDs NO 17 and 16, or SEQ IDs NO 17 and 19, or SEQ IDs NO 26 and 14, or SEQ IDs NO 27 and 28, or SEQ IDs NO 29 and 22, or SEQ IDs NO 32 and 39, or SEQ IDs NO 30 and 18, or SEQ IDs NO 36 and 38, or SEQ IDs NO 37 and 35, or SEQ IDs NO 40 and 25, or SEQ IDs NO 41 and 31, or SEQ IDs NO 42 and 43.

7. A composition comprising at least one nucleic acid molecule according to any of claims 1 to 4 and/or a set of two polynucleotide probes according to claim 5 or claim 6.

8. Use of a nucleic acid molecule consisting of SEQ ID NO 1 or 2, or of the RNA form of said SEQ ID NO 1 or 2 wherein T is replaced by U, or of the complementary form of said SEQ ID NO 1or 2, or of a fragment of at least 20 contiguous nucleotides thereof, or of any of their homologues, for the detection and identification of *Staphylococcus* species, in particular of *S. aureus*.

9. A method for detecting and identifying *Staphylococcus* species using at least one nucleic acid molecule according to any of claims 1 to 4.

10. A method according to claim 9 for detection and identification of a *Staphylococcus* species in a sample comprising the steps of:

(i) if need be releasing, isolating and/or concentrating the polynucleic acids in the sample;

(ii) if need be amplifying the 16S-23S rRNA spacer region, or a fragment comprising the target sequence, or the target sequence or a fragment thereof, with at least one suitable primer pair;

(iii) hybridizing the polynucleic acids of step (i) or (ii) with at least one polynucleotide probe that hybridizes to the target sequence,

wherein the target sequence of step (ii) and (iii) consists of SEQ ID NO 1 or 2 or homologues thereof, or to their RNA form wherein T is replaced by U, or to their complementary form, or a to a fragment of at least 20 contiguous nucleotides thereof,

(iv) detecting the hybrids formed, and

(v) interpreting the signal(s) obtained and inferring the presence of *Staphylococcus* species and identifying the *Staphylococcus* species in the sample.

11. A method according to claim 10 wherein a suitable primer pair consists of any combination of a forward primer polynucleotide selected from the group consisting of SEQ ID NO 45, 49, 50, 52, 56, 61, 63, 64, 65, 66, 67, 68 and their homologues, and a reverse primer polynucleotide selected from the group consisting of SEQ ID NO 46, 47, 48, 51, 53, 54, 55, 57, 58, 59, 60, 62, and their homologues.

12. A method according to claim 10 or claim 11 wherein two polynucleotide probes are used.

13. A method according to claim 12 wherein the two polynucleotide probes hybridize to the target sequence adjacent to each other with less than 25 nucleotides in between.

14. A method according to claim 13 wherein the two polynucleotide probes consist of polynucleotides of SEQ IDs NO 15 and 20, or 15 and 21, or 17 and 16, or 17 and 19, or 26 and 14, or 27 and 28, or 29 and 22, or 32 and 39, or 30 and 18, or 36 and 38, or 37 and 35, or 40 and 25, or 41 and 31, or 42 and 43.

15. A kit for detection and identification of *Staphylococcus* species comprising the following components:

- at least one nucleic acid molecule according to any of claims 1 to 4 and/or a set of two polynucleotide probes according to claim 5 or 6.
- a hybridization buffer, or components necessary for producing said buffer.